patients. However, vaccine efficacy is limited by effector cell dysfunction and increased presence of regulatory T cells characteristic of cancer patients. Ligation of CD3/CD28 delivers an antigen-independent stimulus to resting T cell populations. We postulated that stimulation with DC/tumor fusions followed by anti-CD3/CD28 would result in the significant expansion of activated T cells targeting tumor antigens. DCs were generated from adherent mononuclear cells cultured with rhIL-4, GM-CSF and TNFα and fused with RCC by coculture in 50% solution of polyethylene glycol. T cells were stimulated by DC/tumor fusions prior to or following exposure to antiCD3/CD28 antibody coated plates for 48 hours. A dramatic, statistically significant, increase in T cell proliferation was observed following the sequential exposure to DC/RCC fusions and anti-CD3/CD28 (SI 13.2). In contrast, stimulation by anti-CD3/CD28, DC/tumor fusions, or anti-CD3/CD28 followed by fusion cells did not result in significant T cell proliferation. Similarly, sequential stimulation by DC/RCC fusion cells followed by anti-CD3/CD28 resulted in a nearly 8 fold expansion of CD4+/CD25+ cells (n=10, p=0.001 compared to unstimulated T cells). A 16 fold increase in CD4/CD25/CD69+ cells was observed consistent with the expansion of activated T cells. In contrast, exposure to anti-CD3/CD28 alone or anti-CD3/CD28 followed by stimulation with fusion cells resulted in a 3 fold expansion of CD4/CD25+ T cells and a modest expansion of CD4/CD25/CD69+ cells. In concert with these findings, IFNγ production by CD4+ T cells was most pronounced following stimulation with DC/tumor fusions and expansion with anti-CD3/CD28 ($p<.01$). In 9 experiments, stimulation with DC/RCC fusions followed by expansion with anti-CD3/CD28 also resulted in a 5-fold and 4.6 fold expansion of CD4/CD25/Foxp3+ and IL-10 expressing T cells, respectively. In conclusion, we have demonstrated that stimulation of T cells by DC/RCC fusions followed by exposure to anti-CD3/CD28 antibodies results in the expansion of tumor reactive T cells that predominantly express markers of activation. We are developing a clinical trial in which patients will receive fusion/CD3/CD28 expanded T cells following in vivo depletion of regulatory T cells.

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γδ T CELLS AS IMMUNOTHERAPY FOR GLIOBLASTOMA MULTIFORME

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Background: Despite advances in therapy, the survival for glioblastoma multiforme (GBM) has remained unchanged for 50 years. We have investigated a cellular therapeutic therapy based on innate immune recognition of GBM using *ex vivo* expanded γδ T cells that are highly cytotoxic to GBM lines. Unlike αβ+CD8+ cytotoxic T lymphocytes, γδ T cells act directly against stress-associated antigens expressed on GBM and do not require MHC antigen recognition.

Methods: We examined the circulating γδ T cell number and function in healthy controls and patients at specific times; diagnosis, 1-14 days post resection, and 8-14 weeks post resection. Absolute lymphocyte and subset counts including γδ T cell counts were examined using flow cytometry. Functional response of γδ T cells was determined by proliferation in our clinically compliant expansion procedure and subsequent *in vitro* and *in vivo* cytotoxicity assays. Expansion cultures were harvested after two weeks and enriched for γδ T cells by immunomagnetic depletion of CD4+ and CD8+ T cells. Cytotoxicity of expanded γδ T cells was evaluated *in vitro* against GBM primary tumor cultures, established GBM cell lines and cultured astrocytes. *In vivo* assays were conducted in athymic nude mice against new and established luciferase-transduced human U251 GBM xenografts.

Results: At diagnosis, the circulating γδ T cell count is not significantly less than controls ($p = 0.12$). The absolute CD3 and CD4 count increase immediately after resection, likely due to removal of GBM-derived immunosuppressive cytokines. Surprisingly however, individual patients show a decrease in γδ T cell counts at this time. Expansion of γδ T cells from pre-resection GBM patients did not differ from controls ($p = 0.32$, n=4). although γδ T cell expansion was significantly impaired after cytoreductive therapy ($p = .007$, n=5). Expanded γδ T cells retain cytotoxicity against U251 and 1047 GBM cell lines and GBM primary cultures but spare normal astrocytes. Expanded γδ T cells slow the growth of U251 intracranial xenografts in athymic nude mice ($p = .008$) vs sham-treated controls. Preliminary observations suggest that growth of established U251 xenografts is slowed in selected animals as well.

Conclusions: GBM and therapy-induced immunosuppression present a formidable barrier to systemic cellular therapy. However, intracranial immunotherapy using expanded allogeneic γδ T cells represents a potentially effective immunotherapeutic strategy against GBM.

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SEMI-HIGH-DOSE AUTOLOGOUS AND A NON-MYELOABLATIVE REDUCED INTENSITY CONDITIONING ALLOGENEIC TRANSPLANTS INTEGRATED IN STANDARD OR DOSE DENSE THERAPY FOR BREAST CANCER: COST-EFFICIENCY ANALYSES SUPPORT DESIGN OF COST-EFFICIENT THERAPIES

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Introduction: DFS at 5 yr in LN+ BrCa depends on status at diagnosis and is on average 60% and in MBrCa 15%. HD-auto-Tx add significant DFS advantage of ~ 5% to 10% but statistically this is not a significant improvement of survival. In large series TRM of HD-auto-Tx is on average 3% in the adjuvant and up to 10% in the metastatic setting. Semi-HD is rarely associated with TRM. Allogeneic transplants (allo-Tx) induce an immune response in BrCa that may eradicate minimal residual disease if the Tx is administered in molecular remission. Based on these facts and existent collaborative patterns we designed in 2000 a treatment plan consisting of dose dense induction, two semi-HD-auto-Tx and a NMRICTx and decided to perform upfront cost computation.

Methods: In the Netherlands a cost system is used consisting of so named "diagnose behandelingscombinaties" (DBC) or diagnosis treatment combinations. In general costs are to be collected for each item or service independently. We used the therapeutic model described here above to compute the costs of treatment and used assumption based on literature data to define disease outcome at 5 yr for premenopausal women with ER+, her-2-neu negative BrCa. For each therapy median DFS was used to define outcomes. Disease outcomes were defined as alive without disease, alive with disease, death and toxic death. Utilities for treatment and outcome were derived from literature; The approach followed our Markov model design of 2001.

Results: Disease outcomes at 5 yr of LN + BrCa by respectively 4 cycles induction ChT (1), two semi-HD- auto-Tx (2) and a non-myeloablative reduced intensity conditioning Tx (3) with donor were anticipated to be 60%, 70% and 90% without and 15% with relapse. DBC were identified and included costs for first, second and third line ChT on outpatient basis, or with a hospital episode, costs of hormonal therapy, of a visit without procedure, of auto- and allo- stem cell mobilization and leukapheresis, of auto-and allo-Tx, of post Tx care, of follow-up, of terminal care. We adjusted for the fact that the DBC costs are based on HD- and ablative ChT whereas our approach uses semi-HD and NMRIC respectively. The computations of the cost per treatment with follow-up of five years showed that standard/dose dense ChT followed by hormonal therapy adds up to > 100,000 and that Tx types add each > 50,000 by use of adjusted rates for HD-/ablative Tx.

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THE ROLE OF HUMAN CYP2B6 POLYMORPHISM IN THE BIOACTIVATION OF CYCLOPHOSPHAMIDE USING CDNA EXPRESSED ENZYMES

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